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RESEARCH ARTICLE

Low-frequency electromagnetic fields increase oxidative stress in tobacco plants

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ABSTRACT

Increasing interest on biological effects of electromagnetic radiation, particularly extremely-low-frequency electromagnetic fields (ELF-EMFs) has been observed. Although harmful effects of magnetic fields in humans remain to be demonstrated, it has been proposed that oxidative stress may represent one of the causes of ELF- EMFs adverse effects. We investigated the influence of ELF-EMFs on enzyme activity in tobacco plants under oxidative stress. *Nicotiana tabacum* (L.) seedlings of the Xanthi variety were continuously exposed to 2.0 mT and 60 Hz frequency ELF-EMFs for 24, 48, 72, and 96 h. The biochemical endpoints measured involved leaf and root extracts ascorbate peroxidase (APX) and catalase (CAT) activities, which were increased in leaf extracts after 48, 72, and 96 h of magnetic field exposure, whereas APX and CAT activities in root extracts increased after 48 and 72 h and 48, 72, and 96 h of continuous exposure to magnetic fields. In contrast, ELF-EMF exposure for 24 h did not alter APX and CAT activities in leaf and root extracts. All treatment regimens were matched with a properly sham-exposed control. These results suggest that ELF-EMFs induce an oxidative stress, which may potentiate the oxidative defense system in tobacco plants.

Keywords: Electromagnetic fields; Oxidative stress; Ascorbate peroxidase; Catalase; Tobacco plant

INTRODUCTION

A plethora of studies have been focused on the biological effects of extremely-low-frequency electromagnetic fields (ELF-EMFs). In most cases, the mechanisms of interaction, which are associated with low and moderate flux density magnetic fields, remain to be elucidated. These mechanisms are mostly related to magnetic interactions with enzyme catalyzed-biochemical processes. In addition, it has been reported that magnetic fields are related to oxidative stress (Zhang et al., 2017; Coballase-Urrutia et al., 2018) and antioxidant systems (Scaiano et al., 1994; Li and Chow, 2001; Kula et al., 2002; Zhang et al., 2003) in living organisms.

Increased oxidative enzyme activity on plants by ELF-EMFs and static magnetic fields (SMFs) has been also evidenced (Tkalec et al., 2007; Sharma et al., 2009; Nabizadeh et al. 2014; Jouni et al., 2012). Mahmood et al. (2013) demonstrated significant increase on peroxidase activity after exposing oil palm for six months or seven years to electromagnetic fields from a 275 kV high-voltage transmission line.

On the other hand, tobacco plant offers a versatile model to evaluate physiological changes induced for a variety of factors (Ganapathi et al., 2004), for which we selected this plant model to evaluate EMFs effects on enzyme activities of oxidative stress. In this regard, Sahebjamei et al. (2007) showed increased superoxide dismutase activity and decreased catalase (CAT) and ascorbate peroxidase (APX) activities by 10 mT and 30 mT SMFs exposure of suspension-cultured tobacco cells. They also found increased lipid peroxidation by magnetic fields exposure, indicating that SMFs affected plant cells antioxidant defense mechanism. Moreover, Touati et al. (2013) observed that SMFs at 100 mT exposure treatment on *Raphanus sativus* significantly increased catalase activity in radish plantlets.

Because of magnetic fields are ubiquitous abiotic stressors, they alter life bodies in general. For instance, EMFs have been shown to affect agriculturally important plants (Angel

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et al., 2005). Based in the increasing research regarding this concern, it has been proposed that oxidative stress underlies EMFs effects on plants (Tkalec et al.2005; Gill and Tuteja, 2010; Serdyukov and Novitskii, 2013). On the other hand, science faces the serious challenge of doubling global crop production by 2050, due to the continuing increase in world population and to meet future food demand, changes in diet, and increased consumption of biofuels (Ray et al., 2013). To solve this problem, research has been conducted on different topics in agronomy, particularly the effects of EMFs on plants growth (Pietruszewski and Martínez, 2015).

Taken together, the aim of present study was to evaluate the effect of ELF-EMFs on oxidative stress enzyme activity of tobacco plants.

MATERIALS AND METHODS

Tobacco plants and growth conditions

Tobacco seeds (Xanthi variety of *Nicotiana tabacum* L.) were obtained from the Centro de Investigación y Estudios Avanzados at Instituto Politécnico Nacional, Unidad Irapuato, Guanajuato, México. They were soaked in 70% ethanol for one minute, 20% NaOCI for 20 min, and rinsed with sterile distilled water for 30 min, after which they were planted in Petri dishes (15 seeds/dish), containing MS culture medium (Murashige and Skoog, 1962) and allocated in an environmental chamber (Biotronette Mark III, Lab-Line Instruments, Melrose Park, IL) maintained at 25 ± 0.5 °C undisturbed. We selected seedlings with straight primary roots (21 days aged) for the bioassays and used 15 plantlets for each studied group. Before germination experiments, we determined tobacco seeds viability by the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) colorimetric reduction assay. For this, seeds were soaked in distilled water for 24 h, after which water was removed. Next, a 1% MTT solution was added, and seeds were covered and incubated for 3 d at room temperature in darkness. After this incubation period, the topographic staining pattern was demonstrated with aid of a stereoscopic microscope and the viability percentage was estimated (95% seed-viability was observed). ELF-EMFs treatment regimens are detailed below.

Magnetic field experiments

We used a standardized and calibrated magnetic fields facility, as previously reported (Heredia-Rojas et al., 2004; Heredia-Rojas et al., 2010; Heredia-Rojas et al., 2018). For this, we prepared a coil by winding 552 turns of enamel-insulated copper wire (1.3 mm diameter), which formed a 13.5-cm radius and 71-cm length cylindrical solenoid, which was connected to step-down and variable transformers and plugged to a 110 V AC source. We then placed seedlings in the middle of this device at 2.0 mT homogeneous magnetic field zones at 25 oC ± 0.2 oC and 45% humidity, using magnetic fields (off mode) sham-treated seedlings as negative controls.

We determined the magnetic flux density using a Gaussmeter (Bell FW 6010, Orlando, FL) and an attached oscilloscope (BK-Precision model 2120; Dynascan Corp., Chicago, IL), which was required to measure the resulting field, generating almost pure 60 Hz (< 2% total harmonic distortion) and 0.3 μT and 20 μT values for background magnetic and local geomagnetic fields respectively.

Experimental protocol

We performed the experiments with tobacco seedlings in the presence or absence (sham) of continuous exposure of ELF-EMFs at 60 Hz frequency and 2.0 mT of magnetic flux density, for 24, 48, 72, and 96 h and selected 15 seedlings to evaluate APX and CAT activities in root and leaf extracts.

Enzyme activity assays

We separately homogenized 200 mg (fresh weight) of leaf and root samples in one milliliter of 50 mM Na-phosphate buffer at pH 7.0, containing 5 mM ascorbate, 5 mM dithiothreitol (DTT), 5 mM EDTA,100 mM NaCl, and 2% (w/v) polyvinylpyrrolidone (PVP). Homogenates were filtered through a sterile gauze and filtrate was centrifuged at 15,000 g for 15 min at 4 °C. Supernatant fluids were recovered to measure enzyme activities. In addition, protein contents were evaluated by spectrometry [Bradford method (1976)] at 280 nm, using as a standard, bovine serum albumin.

APX activity was measured according to Nakano and Asada´s method (1981) by determining the ascorbate oxidation rate at 290 nm. The reaction mixture contained 50 mM phosphate buffer at pH 7.0, 0.1 mm EDTA, 5 mM ASH, and 10 μL of the enzyme solution to a final volume of 200 μL, which was initiated by adding 1 mM H2O2. The reaction rate was determined by a reduction in absorbance at 290 nm, whereas the rate constant was calculated using the ASH extinction coefficient of per min-1 mg-1 of protein present and corrected for the rate obtained before H2O2 addition. APX activity was expressed as units of enzyme activity (U), where 1 U is the amount of enzyme in mg that is needed to oxidize one micromol of ascorbic acid to ascorbate during 1 min.

CAT activity was determined by the method of Beers and Sizer (1952), using a reaction mixture containing 50 mM potassium phosphate buffer at pH 7.0 and 10 μL of enzyme solution in 200 μL final volume. The reaction was

initiated by adding 5 mM H2O2, whose decomposition was followed by a decrease in absorbance at 240 nm. CAT activity was expressed as units of enzyme activity (U), where 1 U is the amount of enzyme in mg that is needed to oxidize one micromol of H2O2 during 1 min.

Statistical analysis

Statistical differences for APX and CAT activities between treated cultures and sham-exposed controls were calculated by a *t*-test for independent samples, whereas data normality was calculated by the Kolmogorov-Smirnov test ($p < 0.05$). For these analyses we used the SPSS software version 20.0 (SPSS Inc., Chicago, IL).

RESULTS

Significant ($p < 0.05$) increase in APX activity in tobacco seedlings leaf extracts was observed at 48, 72, and 96 h of continuous ELF-EMFs exposure at 2.0 mT and 60 Hz frequency, as compared with sham-exposed controls ($p < 0.05$) (Fig. 1). Similarly, APX activity was significantly ($p < 0.05$) increased at 48 and 72 h exposure to ELF-EMFs but it was not altered after 24 and 96 h exposure, as compared with sham-exposed cultures ($p > 0.05$).

We also observed a significant ($p < 0.05$) increase in CAT activity in tobacco leaf extracts after 48, 72, and 96 h exposure to 2.0 mT and 60 Hz magnetic fields (Fig. 3). However, we did not find differences at 24 h continuous exposure, as compared with sham-exposed control cultures ($p > 0.05$). In addition, we showed significant ($p < 0.05$) increase of CAT activity in tobacco root extracts after 48, 72, and 96 h exposure to ELF-EMFs (Fig. 4). However, we did not find significant ($p > 0.05$) alterations after 24 h exposure, as compared with sham-exposed controls.

DISCUSSION

In the present study, we determined the effect of 60 Hz and 2.0 mT ELF-EMFs on oxidative stress enzyme activities of tobacco plants. Our results showed that magnetic fields increased APX and CAT activities in leaf and root extracts after 48, 72, and 96 h exposure, which evidenced the plant response to magnetic field exposure-oxidative stress. This is important to elucidate the mechanism of magnetic fields on life bodies. Since the 1970s, oxidative stress has been recognized as an inducer of cytotoxicity, for which many studies have reported protective benefits of antioxidants (Schmidt et al., 2015; Xu et al., 2017).

Living organisms are naturally exposed to environmental magnetic fields, which increases the risk of functional

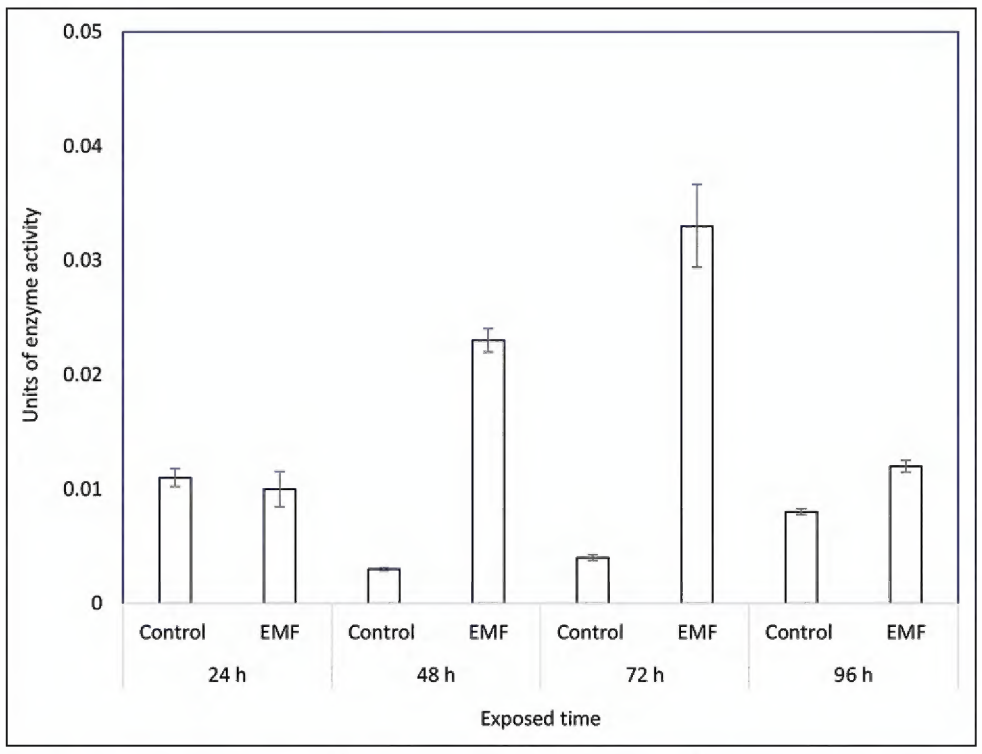

Fig 1. Effect of ELF-EMFs on APX activity in leaf extracts of tobacco seedlings. A significant increased APX activity was observed at 48 h, 72 h, and 96 h of continuous exposure of ELF-EMFs at 60 Hz and 2.0 mT, as compared with sham-exposed controls ($p < 0.05$). Bars represent grouped arithmetical means ± standard error.

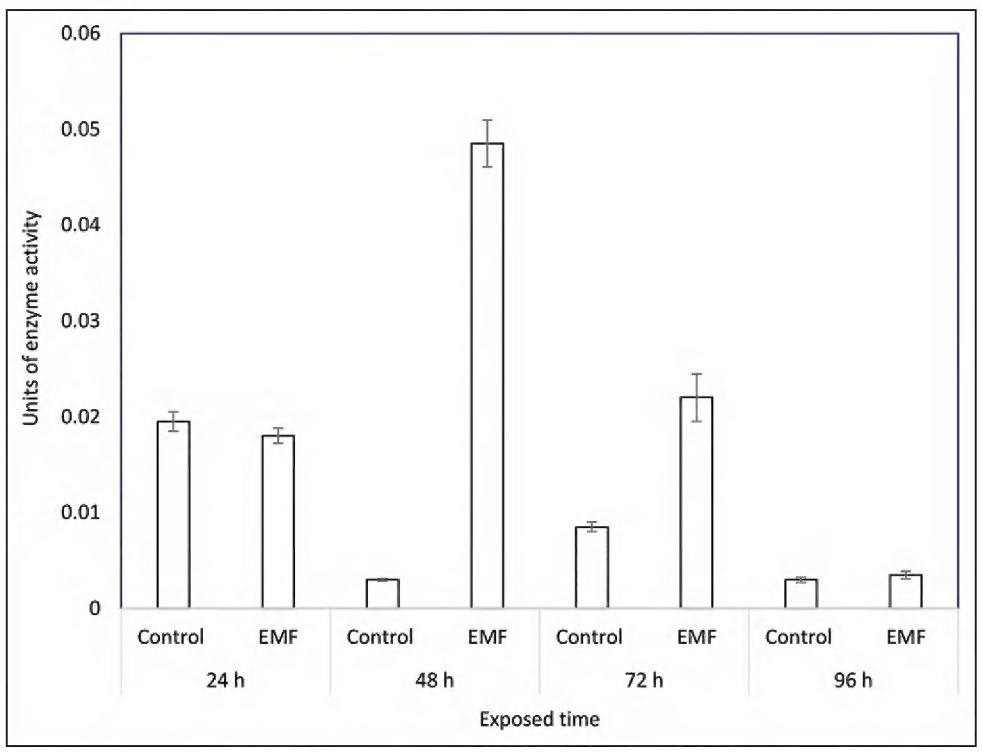

Fig 2. Effect of ELF-EMFs on APX activity values in root extracts of tobacco seedlings. APX activity increased at 48 h and 72 h of continuous exposure of ELF-EMFs at 60 Hz and 2.0 mT ($p < 0.05$) but no difference was found for 24 h and 96 h exposure conditions, as compared with sham-exposed cultures. Bars represent grouped arithmetical means ± standard error.

disorders, particularly in plants that may be also affected by ELF-EMFs sources. Magnetic fields are associated with oxidative stress, which in turn, increases activity, concentration, and lifetime of free radicals, in particular, reactive oxygen species (ROS) (Allen,1995; Mittler,2002; Smirnoff, 1993). Thus, abiotic stress may result in ROS production in plants, causing oxidative stress (Apel and Hirt, 2004). Under stress conditions, we hypothesized that ROS generation is inevitable and plants must produce antioxidant molecules to protect from cellular damage, as previously reported (Foyer and Noctor, 2000; Burritt and MacKenzie, 2003), which is in agreement with our results,

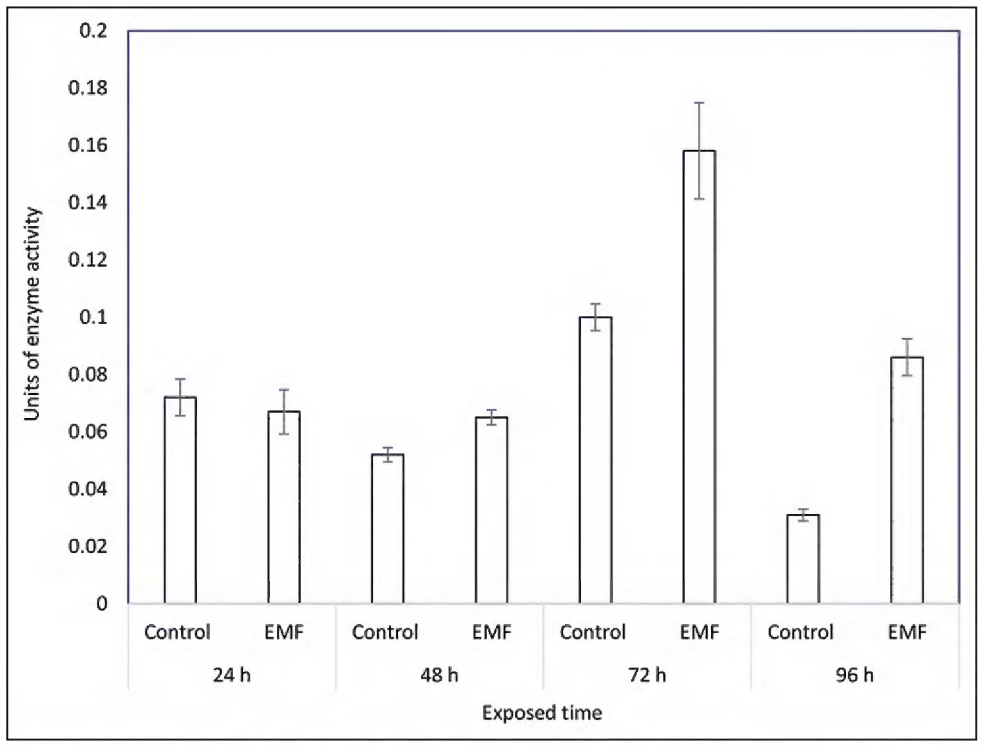

Fig 3. Effect of ELF-EMFs on the CAT enzyme activity in leaf extracts of tobacco seedlings. A statistically ($p < 0.05$) significant increase in CAT activity was observed at 48 h, 72 h, and 96 h of ELF-EMFs exposure. We did not observe differences at 24 h of ELF-EMFs exposure, as compared with control cultures. Bars represent grouped arithmetical means ± standard error.

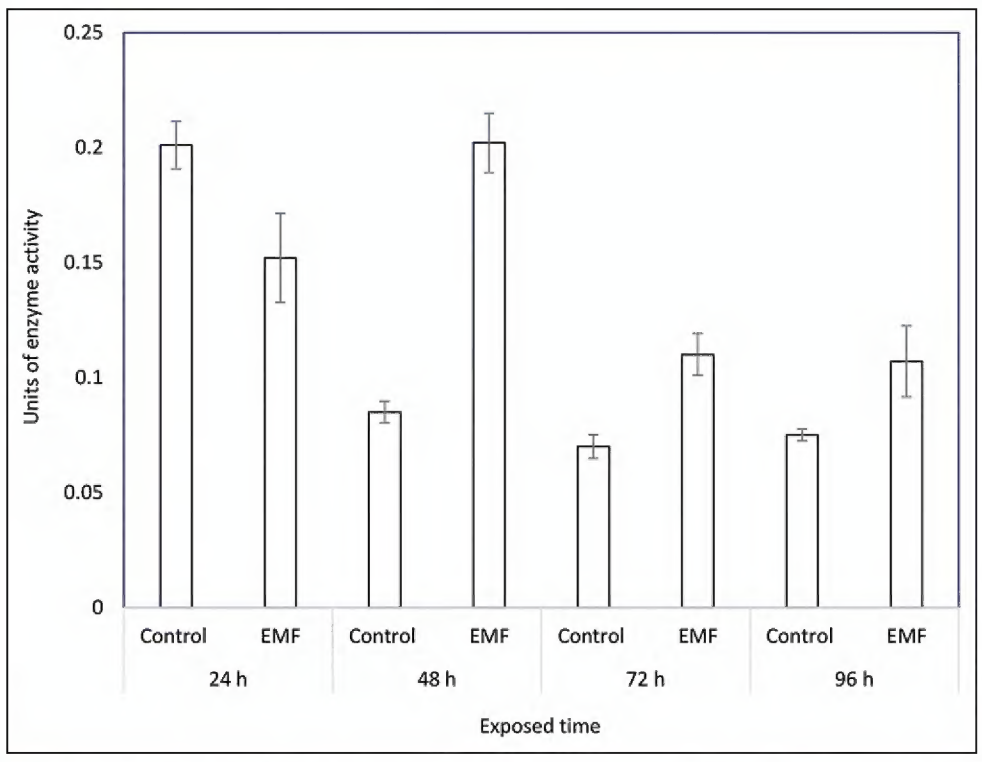

Fig 4. Effect of ELF-EMFs on the CAT enzyme activity in root extracts of tobacco seedlings. A statistically ($p < 0.05$) significant increase in CAT activity was observed at 48 h, 72 h, and 96 h of ELF-EMFs exposure. We did not observe differences at 24 h of ELF-EMFs exposure, as compared with control cultures. Bars represent grouped arithmetical means ± standard error.

showing a significant increase in tobacco plant oxidative stress enzyme activity.

Kthiri et al. (2019) demonstrated that *Saccharomyces cerevisiae* cultures exposure to 250 mT SMFs modified catalase, superoxide dismutase, and glutathione peroxidase activities, thus suggesting an oxidative stress. In this regard, Kıvrak et al. (2017) observed that ELF-EMFs exposure negatively affected antioxidant defense systems, which is relevant considering that oxidative stress develop when an antioxidant defense system does not prevent the harmful effects of free radicals. Moreover, it has been proposed that DNA is indirectly affected by the action of ELF-EMFs, due to anomalous movement of electrons (Valberg et al., 1997) that generate guanine radicals, which by reaction with water causes oxidative DNA damage (Giese, 2006). We have previously demonstrated that ELF-EMFs clastogenicity in mouse bone marrow significantly decreased after animals were pretreated with the antioxidant resveratrol (Heredia-Rojas et al., 2020).

In contrast, several studies have reported anti-oxidative effects of magnetic fields on mammalian cells, particularly ELF-EMFs, similar to those used in our study in tobacco plants (Balind et al., 2014; Cichoń et al., 2017). In addition, Ahn et al. (2020) observed that exposure to pulsed magnetic fields (PMFs) prevented red blood cells from oxidative stress. Regarding mammalian cells, it has been proposed that magnetic fields increase the activity, concentration, and lifetime of free radicals after SMFs (Politański et al. 2010; Ghodbane et al., 2013) or electromagnetic field (Kerimoğlu et al., 2018) exposure.

In conclusion, 2.0 mT and 60 Hz ELF-EMFs exposure induced oxidative stress in tobacco plants, and as a consequence, potentiated their oxidative defense system. Although ELF-EMFs-induced cytotoxicity mechanism has not yet been elucidated, reports have shown intrinsic electrical features associated with biostructures and biological functions. Living organisms are sensitive to external electromagnetic fields of very week intensities, which may be debatable. The lack of objective mechanisms to associate ELF-EMFs exposure and biological events has resulted in unclear investigations, inconsistent observations, and interpretations. To date, we do not have an accepted mechanism by which ELF-EMFs consistently produced an oxidative stress condition. However, there is a consensus among researchers that oxidative stress is one of the main biological effects of magnetic fields. Further studies are still necessary to elucidate its mechanism.

Author´s contributions

J. Antonio Heredia-Rojas: Design of the experiments, bioassays, experimental procedures; Abraham O. Rodríguez-De la Fuente: Conceptualization, data curation and statistical analyses; Erick Freeze-Gallardo: Experimental procedures; Deyanira Quistian-Martínez: Supervision, writing-review and editing; Ricardo Gomez-Flores: Substantially revised the work and original draft preparation; David F. La fuente-Rincón: Experimental procedures, supervision; Omar Heredia-Rodríguez: Magnetic field exposure facilities and measurements and Alberto Valadez-Lira: Critically revising the manuscript and original draft. All authors read and approved the final manuscript.

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